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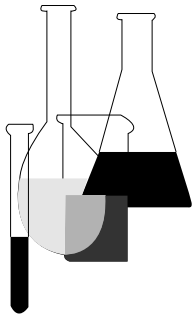
Health Effects Test Guidelines OPPTS 870.7800 Immunotoxicity



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 870.7800 Immunotoxicity.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** This is a new guideline developed in the Office of Pesticide Programs.

(b) **Purpose.** This guideline is intended to provide information on suppression of the immune system which might occur as a result of repeated exposure to a test chemical. While some information on potential immunotoxic effects may be obtained from hematology, lymphoid organ weights and histopathology (usually done as part of routine toxicity testing), there are data which demonstrate that these endpoints alone are not sufficient to predict immunotoxicity (Luster *et al.*, 1992, 1993 see paragraphs (j)(8) and (j)(9) of this guideline). Therefore, the tests described in this guideline are intended to be used along with data from routine toxicity testing, to provide more accurate information on risk to the immune system. The tests in this guideline do not represent a comprehensive assessment of immune function.

(c) **Definitions.** The following definitions apply to this guideline.

Antibodies or immunoglobulins (Ig) are part of a large family of glycoprotein molecules. They are produced by B cells in response to antigens, and bind specifically to the eliciting antigen. The different classes of immunoglobulins involved in immunity are IgG, IgA, IgM, IgD, and IgE. Antibodies are found in extracellular fluids, such as serum, saliva, milk, and lymph. Most antibody responses are T cell-dependent, that is, functional T and B lymphocytes, as well as antigen-presenting cells (usually macrophages), are required for the production of antibodies.

CD is the abbreviation for *cluster of differentiation*, and refers to molecules expressed on the cell surface. These molecules are useful as distinct CD molecules are found on different populations of cells of the immune system. Antibodies against these cell surface markers (e.g., CD4, CD8) are used to identify and quantitate different cell populations.

Immunotoxicity refers to the ability of a test substance to suppress immune responses that could enhance the risk of infectious or neoplastic disease, or to induce inappropriate stimulation of the immune system, thus contributing to allergic or autoimmune disease. This guideline only addresses potential immune suppression.

Natural Killer (NK) cells are large granular lymphocytes which nonspecifically lyse cells bearing tumor or viral antigens. NK cells are upregulated soon after infection by certain microorganisms, and are thought to represent the first line of defense against viruses and tumors.

T and B cells are lymphocytes which are activated in response to specific antigens (foreign substances, usually proteins). B cells produce antigen-specific antibodies (see the definition for ‘‘antibodies or immunoglobulins’’), and subpopulations of T cells are frequently needed to provide help for the antibody response. Other types of T cell participate in the direct destruction of cells expressing specific foreign (tumor or infectious agent) antigens on the cell surface.

(d) **Principles of the test methods.** (1) In order to obtain data on the functional responsiveness of major components of the immune system to a T cell dependent antigen, sheep red blood cells (SRBC), rats and/or mice[1] must be exposed to the test and control substances for at least twenty eight days.[2] The animals must be immunized by intravenous or intraperitoneal injection of SRBCs approximately four days (depending on the strain of animal) prior to the end of the exposure. At the end of the exposure period, either the plaque forming cell (PFC) assay or an enzyme linked immunosorbent assay (ELISA) must be performed to determine the effects of the test substance on the splenic anti-SRBC (IgM) response or serum anti-SRBC IgM levels, respectively.

(2) In the event the test substance produces significant suppression of the anti-SRBC response, expression of phenotypic markers for major lymphocyte populations (total T and total B), and T cell subpopulations (T helpers (CD4) and T cytotoxic/suppressors (CD8)), as assessed by flow cytometry, may be performed to determine the effects of the test substance on either splenic or peripheral-blood lymphocyte populations and T cell subpopulations. When this study is performed, the appropriate monoclonal antibodies for the species being tested should be used. If the test substance has no significant effect on the anti-SRBC assay, a functional test for NK cells may be performed to test for a chemical’s effect on non-specific immunity.[3] For tests performed using cells or sera from blood (ELISA or flow cytometry), it is not necessary to destroy the animals, since immunization with SRBCs at twenty eight days is not expected to markedly affect the results of other assays included in subchronic or longer-term studies (Ladics *et al*., 1995 see paragraph (j)(7) of this guideline). The necessity to perform either a quantitative analysis of the effects of a chemical on the numbers of cells in major lymphocyte populations and T Cell subpopulations by flow cytometry, or a splenic NK cell activity assay to assess the effects of the test compound on non-specific immunity should

[1] If absorption/distribution/metabolism/excretion (ADME) data are similar between species, then either rats or mice may be used for the test compound in question. If such data are lacking, both species should be used.

[2] Because there is a fairly rapid turnover of many of the cells in the immune system, twenty eight days is considered sufficient for the purposes of the anti-SRBC tests.

[3] When these optional tests are included, the phenotypic or NK cell analyses may be performed at twenty eight days of exposure, or at a later timepoint if ADME data suggest that a longer exposure is more appropriate.

be determined on a case-by-case basis, depending upon the outcome of the anti-SRBC assay.

(e) **Limit test.** If a test at one dose level of at least 1,000 mg/kg body weight (or 2 mg/L for inhalation route of exposure) using the procedures described for this study produces no observable toxic effects or if toxic effects would not be expected based upon data of structurally related compounds, then a full study using three dose levels might not be necessary. Expected human exposure may indicate the need for a higher dose level.

(f) **Test procedures**—(1) **Animal selection**—(i) **Species and strain.** These tests are intended for use in rats and/or mice. Commonly used laboratory strains should be employed.[4] All test animals should be free of pathogens, internal and external parasites. Females should be nulliparous and nonpregnant. The species, strain and source of the animals must be identified.

(ii) **Age/weight.** (A) Young, healthy animals should be employed. At the commencement of the study, the weight variation of the animals used should not exceed ± twenty percent of the mean weight for each sex.

(B) Dosing should begin when the test animals are between six and eight weeks old.

(iii) **Sex.** Either sex may be used in the study; if one sex is known or believed to be more sensitive to the test compound, then that sex should be used.

(iv) **Numbers.** (A) At least eight animals should be included in each dose and control group. The number of animals tested should yield sufficient statistical power to detect a twenty percent change based upon the interanimal variation which may be encountered in these assays.

(B) To avoid bias, the use of adequate randomization procedures for the proper allocation of animals to test and control groups is required.

(C) Each animal should be assigned a unique identification number. Dead animals, their preserved organs and tissues, and microscopic slides should be identified by reference to the animal's unique number.

(v) **Husbandry.** (A) Animals may be group-caged by sex, but the number of animals per cage must not interfere with clear observation of each animal. The biological properties of the test substance or toxic effects (e.g., morbidity, excitability) may indicate a need for individual caging.

[4] The study director should be aware of strain differences in response to SRBC. For example, if the $B_6C_3F_1$ hybrid mouse is used in the PFC assay, a response of 800–1,000 PFC/10^6 spleen cells in control mice should be the minimally acceptable PFC response.

(B) The temperature of the experimental animal rooms should be at 22 ± 3°C.

(C) The relative humidity of the experimental animal rooms should be between thirty and seventy percent.

(D) Where lighting is artificial, the sequence should be 12 hours light, 12 hours dark.

(E) Control and test animals should be maintained on the same type of bedding and receive feed from the same lot. The feed should be analyzed to assure adequacy of nutritional requirements of the species tested and for impurities that might influence the outcome of the test. Rodents should be fed and watered *ad libitum* with food replaced at least weekly.

(F) The study should not be initiated until the animals have been allowed an adequate period of acclimatization or quarantine to environmental conditions. The period of acclimatization should be at least one week in duration.

(2) **Control and test substances.** (i) Where necessary, the test substance should be dissolved or suspended in a suitable vehicle. Ideally, if a vehicle or diluent is needed, it should not elicit toxic effects or substantially alter the chemical or toxicological properties of the test substance. It is recommended that an aqueous solution should be used. If solubility is a problem a solution in oil may be used. Other vehicles may be considered, but only as a last resort.

(ii) One lot of the test substance should be used, if possible, throughout the duration of the study, and the research sample should be stored under conditions that maintain its purity and stability. Prior to the initiation of the study, there should be a characterization of the test substance, including the purity of the test compound and if technically feasible, the name and quantities of any known contaminants and impurities.

(iii) If the test or positive control substance is to be incorporated into feed or another vehicle, the period during which the test substance is stable in such a mixture should be determined prior to the initiation of the study. Its homogeneity and concentration must also be determined prior to the initiation of the study and periodically during the study. Statistically randomized samples of the mixture should be analyzed to ensure that proper mixing, formulation, and storage procedures are being followed, and that the appropriate concentration of the test or control substance is contained in the mixture.

(3) **Control groups.** (i) A concurrent, vehicle-treated control group is required.

(ii) A separate untreated control group is required if the toxicity of the vehicle is unknown.

(iii) A positive control group with a known immunosuppressant (e.g., cyclophosphamide) is useful in the interpretation of the results or verification of the assay sensitivity, and should be included in the study. When used, a group of at least eight animals should be given the immunosuppressive chemical.

(4) **Dose levels.** (i) In repeated-dose toxicity tests, it is desirable to have a dose-response relationship and a no observed immunotoxic effect level. Therefore, at least three dose levels and a negative control should be used, unless a Limit test is performed as specified in paragraph (e) of this guideline.

(ii) The highest dose level should not produce significant stress, malnutrition, or fatalities, but ideally should produce some measurable sign of general toxicity (e.g., a ten percent loss of body weight).

(iii) The lowest dose level ideally should not produce any evidence of immunotoxicity.

(5) **Administration of the test substance.** (i) The test substance, vehicle, or positive control substance shall be administered for at least twenty eight days for the anti-SRBC assay. The route of administration of the test material will usually be oral; however, this should be determined by the likely route of occupational or indoor exposure. Therefore, under certain conditions, the dermal or inhalation route of exposure may be more relevant for the study. All animals should be dosed by the same method during the entire experimental period.

(ii) If the test substance is administered by gavage, the animals are dosed with the test substance ideally on a seven-days-per-week basis. However, based primarily on practical considerations, dosing by gavage on a five-days-per-week basis is acceptable. If the test substance is administered in the drinking water, or mixed directly into the diet, then exposure should be on a seven-days-per-week basis.

(A) For substances of low toxicity, it is important to ensure that when administered in the diet, the quantities of the test substance involved do not interfere with normal nutrition. When the test substance is administered in the diet, either a constant dietary concentration in parts per million (ppm) or a constant dose level in terms of the animal's body weight should be used; the alternative used must be specified.

(B) For a substance administered by gavage, the dose should be given at approximately the same time each day, and adjusted at intervals (weekly for mice, twice per week for rats) to maintain a constant dose level in terms of the animal's body weight.

(iii) If the test substance is administered dermally, refer to the Health Effects Test Guidelines, OPPTS 870.3250, Subchronic Dermal Toxicity,

paragraphs (e)(5), (e)(6), (e)(7), and (e)(8) for the procedures to be used. The exposure time for the anti-SRBC test should be at least twenty eight days.

(iv) If the test substance is administered by the inhalation route, refer to the Health Effects Test Guidelines, OPPTS 870.3465, Subchronic Inhalation Toxicity, paragraphs (e)(2), (e)(3), (e)(6), (e)(8), (e)(9), and (e)(10) for the procedures to be used. The exposure time for the anti-SRBC test should be at least twenty eight days.

(6) **Observation period.** Duration of the observation period should be at least twenty eight days.

(7) **Observation of animals.** (i) Observations should be made at least once each day for morbidity and mortality. Appropriate actions should be taken to minimize loss of animals to the study (e.g., necropsy of those animals found dead and isolation or euthanasia of weak or moribund animals).

(ii) A careful clinical examination should be made at least once a week. Observations should be detailed and carefully recorded, preferably using explicitly defined scales. Observations should include, but not be limited to: evaluation of skin and fur, eyes and mucous membranes; respiratory and circulatory effects; autonomic effects, such as salivation; central nervous system effects, including tremors and convulsions, changes in the level of motor activity, gait and posture, reactivity to handling or sensory stimuli, grip strength, and stereotypes or bizarre behavior (e.g., self-mutilation, walking backwards).

(iii) Signs of toxicity should be recorded as they are observed, including the time of onset, degree and duration.

(iv) Food and water consumption should be determined weekly.

(v) Animals should be weighed immediately prior to dosing, weekly (twice per week for rats) thereafter, and just prior to euthanasia.

(vi) Any moribund animals should be removed and euthanized when first noticed. Necropsies should be conducted on all moribund animals, and on all animals that die during the study.

(vii) The spleen and thymus should be weighed in all animals at the end of the study.

(g) **Immunotoxicity tests**—(1) **Functional tests.** Either a splenic PFC assay or an ELISA must be used to determine the response to antigen administration.

(i) **Antibody plaque-forming cell (PFC) assay.** The Jerne and Nordin antibody plaque-forming cell assay, as modified by Cunningham

(1965) (see paragraph (j)(2) of this guideline) or as described in detail by Holsapple (1995) (see paragraph (j)(4) of this guideline), should be used to demonstrate the effects of exposure (at least twenty eight days) to a test substance on antibody-producing cells from the spleen. The following points should be adhered to when conducting this assay:

(A) The T cell-dependent antigen, SRBC, should be injected intravenously or intraperitoneally, usually at twenty four days after the first dosing with the test substance.[5] Although the optimum response time is usually four days after immunization, some strains of test animal may deviate from this time point. Hence, the strain to be used must be evaluated for the optimum day for PFC formation after immunization.

(B) The activity of each new batch of complement must be determined. For any given study, the SRBCs should be from a single sheep, or pool of sheep, for which the shelf life and dose for optimum response has been determined.

(C) Modifications of the above-cited PFC assay exist (for example, see Ladics *et al.*, 1994 or Temple, *et al.* 1993 in paragraphs (j)(5), (j)(6), and (j)(10) of this guideline) and may prove useful; however, the complete citation should be made for the method used, any modifications to the method should be reported, and the source and, where appropriate, the activity or purity of important reagents should be given. Justification or rationale should be provided for each protocol modification.

(D) Samples must be randomized and coded for PFC analysis, so that the analyst is unaware of the treatment group of each sample examined.

(E) Spleen cell viability should be determined.

(F) The numbers of IgM PFC per spleen, and the number of IgM PFC per 10^6 spleen cells must be reported.

(ii) **Immunoglobulin quantification.** Enzyme-Linked Immunosorbent Assay (ELISA). As an alternative to a PFC assay, the effects of the test substance on the antibody response to antigen may be determined by an ELISA (see Temple, *et al.*, 1993, and Ladics *et al.*, 1994 in paragraphs (j)(5), (j)(6), and (j)(10) of this guideline for a comparison between the PFC and ELISA assays for immunotoxicity assessment). Test animals must be immunized with SRBCs as for the PFC assay. IgM titers in the serum of each test animal must be determined (usually four days after immunization). As with the PFC assay, the optimum dose of SRBCs and optimum time for collection of the sera must be determined for the species and strain of animal to be tested. Detailed methods are described by Temple *et al.* (1995) (see paragraph (j)(11) of this guideline).

[5] If the SRBCs are administered by the intraperitoneal route, the study director should be aware that a low percentage of animals may not respond because the antigen was accidentally injected into the intestinal tract.

(iii) Natural killer (NK) cell activity. The methods in Djeu, *et al.* (1995) (see paragraph (j)(3) of this guideline) may be used to demonstrate the effects of at least twenty eight days of exposure to a test substance on spontaneous cytotoxic activity. In this assay, splenocytes from treated and untreated test animals are incubated with ^{51}Cr-labeled YAC-1 lymphoma cells. The amount of radiolabel released from the target cells after incubation with the effector cells for four hours is used as a measure of NK cytolysis. The following points should be adhered to when using the NK cell assay:

(A) Assay controls should be included to account for spontaneous release of radiolabel from target cells in the absence of effector cells, and also for the determination of total release of radiolabel.

(B) Target cells other than YAC-1 lymphoma cells may be appropriate for use in the assay. In all cases, target cell viability should be determined.

(C) Modifications of the protocol exist that may prove useful. However, complete citation must be made to the method used. Modifications must be reported, and where appropriate, the source, activity, and/or purity of the reagents should be given. Justification or rationale must be provided for each protocol modification.

(2) **Enumeration of splenic or peripheral blood total B cells, total T cells, and T cell subpopulations.** The phenotypic analysis of total B cell, total T cell, and T cell subpopulations from the spleen or peripheral blood by flow cytometry should be performed after at least twenty eight days of dosing; this may be performed at a later timepoint, if ADME data suggest that a longer exposure is more appropriate. If an exposure period longer than twenty eight days is used, then these tests may be performed in conjunction with subchronic (ninety day oral, dermal, or inhalation) toxicity studies, when these studies are required. Methods are described by Ladics and Loveless (1994), and Cornacoff *et al.* (1995) (see paragraphs (j)(1) and (j)(5) of this guideline).

(h) **Data and reporting**—(1) **Treatment of results**—(i) Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing effects, the types of effects and the percentage of animals displaying each type of effect.

(ii) All observed results, quantitative and incidental, should be evaluated by an appropriate statistical method. Any generally accepted statistical methods may be used; the statistical methods including significance criteria should be selected during the design of the study.

(2) **Evaluation of study results.** The findings of an immunotoxicity study should be evaluated in conjunction with the findings of preceding

studies and considered in terms of other toxic effects. The evaluation should include the relationship between the dose of the test substance and the presence or absence, and the incidence and severity of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects. A properly conducted test should provide a satisfactory estimation of a no-observed-effect level. It also may indicate the need for an additional study and provide information on the selection of dose levels.

(3) **Test report.** In addition to the reporting requirements as specified under EPA Good Laboratory Practice Standards, 40 CFR part 792, Subpart J, 40 CFR part 160, and the OECD principles of GLP (ISBN 92–64–12367–9), the following specific information should be reported:

(i) The test substance characterization should include:

(A) Chemical identification.

(B) Lot or batch number.

(C) Physical properties.

(D) Purity/impurities.

(E) Identification and composition of any vehicle used.

(ii) The test system should contain data on:

(A) Species, strain, and rationale for selection of animal species, if other than that recommended.

(B) Age, body weight data, and sex.

(C) Test environment including cage conditions, ambient temperature, humidity, and light/dark periods.

(D) When inhalation is the route of exposure, a description of the exposure equipment and data should be included (refer to the Health Effects Test Guidelines, OPPTS 870.3465 Subchronic Inhalation Toxicity, paragraphs (f)(3)(iii)(D), and (f)(3)(iii)(E)).

(E) Identification of animal diet.

(iii) The test procedure should include the following data:

(A) Method of randomization used.

(B) Full description of experimental design and procedure.

(C) Dose regimen including levels, methods, and volume.

(iv) Test results—(A) Group animal data. Tabulation of toxic response data by species, strain, sex and exposure level for:

(*1*) Number of animals exposed.

(*2*) Number of animals showing signs of toxicity.

(*3*) Number of animals dying.

(B) Individual animal data. Data should be presented as summary (group mean) as well as for individual animals.

(C) Date of death during the study or whether animals survived to termination.

(D) Date of observation of each abnormal sign and its subsequent course.

(E) Absolute and relative spleen and thymus weight data are required.

(F) Feed and water consumption data, when collected.

(G) Results of immunotoxicity tests.

(H) Necropsy findings of animals that were found moribund and euthanized or died during the study.

(I) Statistical treatment of results, where appropriate.

(i) **Quality control.** A system should be developed and maintained to assure and document adequate performance of laboratory staff and equipment. The study should be conducted in compliance with the Good Laboratory Practice (GLP) regulations as described by the Agency (40 CFR parts 160 and 792) and the OECD principles of GLP (ISBN 92–64–12367–9).

(j) **References.** The following references should be consulted for additional background information on this test guideline:

(1) Cornacoff, J.B., Graham, C.S., and LaBrie, T.K. 1995. Phenotypic identification of peripheral blood mononuclear leukocytes by flow cytometry as an adjunct to immunotoxicity evaluation. In *Methods in Immunotoxicology* (G.R. Burleson, J.H. Dean, and A.E. Munson, Eds.), Vol. 1, pp 211–226, Wiley-Liss, Inc., New York.

(2) Cunningham, A.J. 1965. A method of increased sensitivity for detecting single antibody-forming cells. *Nature* 207:1106–1107.

(3) Djeu, Julie Y. 1995. Natural Killer Activity. In *Methods in Immunotoxicology* (G.R. Burleson, J.H. Dean, and A.E. Munson, Eds.) pp 437–449.

(4) Holsapple, M.P. 1995. The plaque-forming cell (PFC) response in Immunotoxicology: An approach to monitoring the primary effector function of B lymphocytes. In *Methods in Immunotoxicology* (G.R. Burleson, J.H. Dean, and A.E. Munson, Eds.), Vol. 1, pp. 71–108, Wiley-Liss, Inc., New York.

(5) Ladics, G.S. and Loveless, S.E. 1994. Cell surface marker analysis of splenic lymphocyte populations of the CD rat for use in immunotoxicological studies. *Toxicol. Methods* 4: 77–91.

(6) Ladics, G.S., Smith, C., Heaps, K., and Loveless, S.E. 1994. Evaluation of the humoral immune response of CD rats following a 2-week exposure to the pesticide carbaryl by the oral, dermal, or inhalation routes. *J. Toxicol. Environ. Health* 42:143–156.

(7) Ladics., G.S., Smith, C., Heaps, K., Elliot, G.S., Slone, T.W., and Loveless, S.E. 1995. Possible incorporation of an immunotoxicological functional assay for assessing humoral immunity for hazard identification purposes in rats on standard toxicology study. *Toxicology* 96:225–238.

(8) Luster, M.I., Portier, C., Pait, D.G., White, K.L., Jr., Gennings, C., Munson, A.E., and Rosenthal, G.J. 1992. Risk assessment in immunotoxicology I. Sensitivity and predictability of immune tests. *Fundam. Appl. Toxicol.* 18:200–210.

(9) Luster, M.I., Portier, C., Pait, D.G., Rosenthal, G.J. Germolec. D.R., Corsini, E., Blaylock, B.L., Pollock, P., Kouchi, Y., Craig, W., White, D.L., Munson, A.E., and Comment, C.E. 1993. Risk Assessment in Immunotoxicology II. Relationships Between Immune and Host Resistance Tests. *Fundam. Appl. Toxicol.* 21:71–82.

(10) Temple, L., T. T. Kawabata, A. E. Munson, and K. L. White, Jr. 1993. Comparison of ELISA and plaque-forming cell assays for measuring the humoral immune response to SRBC in rats and mice treated with benzo[a]pyrene or cyclophosphamide. *Fundam. Appl. Toxicol.* 21:412–419.

(11) Temple, L., Butterworth, L., Kawabata, T.T., Munson, A.E., and White, K.L. 1995. ELISA to Measure SRBC Specific Serum IgM: Method and Data Evaluation. In *Methods in Immunotoxicology* (G.R. Burleson, J.H. Dean, and A.E. Munson, Eds.), Vol. 1, pp 137–157, Wiley-Liss, Inc., New York.